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Costal-2: A Scaffold for Kinases Mediates Hedgehog Signaling

Drosophila **genetics has identified several components of the Hedgehog signaling pathway, but the mechanism by which they act remains elusive. In this issue of *Developmental Cell*, a report by Zhang et al. provides evidence that the kinesin-related protein Costal-2 forms a multi-component scaffold that mediates Hedgehog signaling.**

The Hedgehog (Hh) signal-transduction pathway plays important roles both in development and in cancer biology (McMahon et al., 2003). Several factors involved in the regulation of Hh signaling have been identified through studies using *Drosophila melanogaster*. The ultimate target of the Hh pathway is the regulation of the transcription factor Cubitus interruptus (Ci)/Glioblastoma (Gli). The transmembrane protein Smoothened (Smo) is essential for cells to transduce a Hh signal, but Smo does not bind to Hh directly. Instead, Hh is a ligand for the 12-transmembrane-domain protein Patched (Ptc). It is believed that Ptc is a negative regulator of Smo and that Ptc binding to Hh initiates a signaling pathway that relieves the inhibitory activity of Ptc on Smo. In the absence of a Hh signal, Ptc is found principally on the surface of cells, whereas Smo is found primarily in intracellular vesicles. In response to Hh stimulation, Ptc is removed from the cell surface by endocytosis. Subsequently, Smo moves to the plasma membrane and is hyperphosphorylated.

Genetic and biochemical studies have indicated that, in the absence of Hh signaling, the kinesin-related protein Costal-2 (Cos2), the serine-threonine protein kinase Fused (Fu), the biochemically uncharacterized Suppressor of Fused (Su(fu)), and Ci form a large protein complex that is attached to microtubules through the Cos2 motor domain (McMahon et al., 2003). In the absence of Hh stimulation, the full-length, transcriptionally competent Ci, Ci-155, is retained in the cytoplasm and is cleaved to the form a transcriptional repressor, the Ci-75 N-terminal fragment of Ci, or is completely degraded. The processing and/or degradation of Ci is regulated by the cAMP-dependent protein kinase (PKA). PKA phosphorylates multiple Ser/Thr residues in the C-terminal half of Ci, and these phosphorylations are essential for proteasome-dependent processing by the F-box-containing protein Slimb (Wang et al., 1999; Chen et al., 1999) and for lysosome-dependent complete degradation via Debra (Dai et al., 2003). Loss of dPKA causes an accumulation of Ci-155, which is competent to activate transcription of Hh target genes. Phosphorylation of Ci-155 by dPKA induces further phosphorylations of the protein Ci by glycogen synthase kinase 3 (GSK3) (Jia et al., 2002) and casein kinase 1 (CK1) (Price and Kalderon, 2002). All of these phosphorylation events appear to be required for the processing of Ci-155 to Ci-75 because *Drosophila* GSK3 mutants, the *zw3/sgg* mutants, show an accumulation of Ci-155. Thus, Ci/Gli phosphorylation and processing are key regulatory events in the Hh signal-transduction pathway.

Previous work has shown that, in response to Hh stimulation, the actions of PKA, GSK3, and CKI are blocked, the processing of Ci is abrogated, and Ci-155 accumulates. However, the precise mechanism by which Hh inhibits Ci phosphorylation by these kinases has remained a mystery. Now, an elegant study by Zhang et al. (2004) in this issue of *Developmental Cell* presents new data that provide the key to understanding this mechanism. The authors have demonstrated that the kinesin-related protein Cos2 forms a complex with three kinases, PKA, GSK3, and CKI, both in vitro and in vivo.

To demonstrate the in vivo interaction, a Kinesin-Cos2 chimeric protein was used. Cos2 contains a microtubule binding domain, but in Cos-2 this domain does not contain the conserved motif essential for kinesin motor function, which argues against a motor function for Cos2. The Kinesin-Cos2 chimera, in which the microtubule binding domain of Cos2 is replaced by a canonical kinesin motor domain, was expected to move along microtubules and carry Cos2-interacting proteins to the microtubule plus end. Accumulation of Kinesin-Cos2 at the plus ends of microtubules provided a discrete intracellular localization that allowed the authors to identify colocalized proteins by immunofluorescence. PKA, GSK3, and CKI, which normally show a diffuse cytoplasmic localization, were, in fact, enriched at the microtubule plus end in cells expressing Kinesin-Cos2, indicating that they colocalized with the chimeric protein. Additionally, the authors showed that Cos2 is required for Ci phosphorylation by these kinases. These data strongly suggest that Cos2 forms a scaffold mediating the interactions between Ci and the three kinases.

Previous studies have demonstrated that Hh induces a direct interaction between Smo and Cos2 (Lum et al., 2003; Jia et al., 2003). The Smo-Cos2 interaction appears to disrupt the Cos2-Ci interaction. Consistent with these data, Zhang et al. demonstrated that the Smo C-terminal tail colocalized with the Kinesin-Cos2 chimeric protein and that the Smo C-terminal tail fragment competed for binding with GSK3 and CKI. Hh signaling also inhibited the interaction between Cos2 and the three kinases. Based on these data, the authors have proposed the following model. Cos2 provides a scaffold for Ci, PKA, GSK3, and CKI. When these components are bound to Cos2 and in close proximity, Ci can be hyperphosphorylated and processed into its Ci-75 transcriptional-repressor form. In the presence of Hh, Smo is disinhibited and binds Cos2, leading to dissociation of the scaffolded components and inhibition of Ci phosphorylation and subsequent processing. Thus, the Ci-155 transcriptional activator accumulates, and Hh target genes are expressed.

Although this elegant study by Zhang et al. has clearly demonstrated the mechanism by which Hh sig-

naling blocks Ci phosphorylation by the three kinases, many questions remain unanswered. Recently, Jiang's group showed that PKA and CKI directly phosphorylate Smo and induce its cell-surface accumulation (Jia et al., 2004). However, it is not yet clear what mechanism links Smo phosphorylation to the transport of Smo to the cell surface or whether the kinases must be scaffolded to Cos2 to mediate Smo phosphorylation. In addition, to induce the Smo-Cos2 interaction, Cos2 may be recruited to membranes, but it is not known whether the association of Cos2 with microtubules regulates this connection. Upon Hh stimulation, Fu is also activated by hyperphosphorylation and, in turn, phosphorylates Cos2. Although the role of Cos2 phosphorylations in modulating Cos2 function is not clear, it is tempting to speculate that the phosphorylation events regulate the binding of the Fu/Cos2/Ci complex to microtubules. Furthermore, a small proportion of Su(fu) becomes phosphorylated, possibly by Fu. Because Su(fu) interacts with SAP18, a component of the histone deacetylase complex Sin3, one interesting possibility is that this phosphorylation event makes the Ci-Su(fu) complex no longer repressive by releasing SAP18. Future work will undoubtedly address these remaining issues and should allow us to bridge the current gaps in the Hh signal-transduction pathway, and as is the case with the report by Zhang et al., every new discovery will remind us of the interesting and elegant complexity of the Hh pathway.

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Selected Reading

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